



# A novel soil amendment for enhancing soil moisture retention and soil carbon in drought-prone soils

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## ABSTRACT

Crop yield reductions are common in drought-stressed agroecosystems and are likely to become more frequent with climate change. To combat this, soil amendments are often used to enhance soil moisture retention but typically only lead to marginal improvements. Moreover, even as concern over agricultural water use mounts, a large fraction of food is wasted. Diverting more food waste and byproducts back to agricultural fields could reduce waste issues while ameliorating critical water limitations. We evaluated lactobionate, a lactose derivative and major dairy industry byproduct, as a potential soil amendment for enhancing both soil moisture and soil organic carbon (SOC). Lactobionate (LB) is a hydrophilic compound consisting primarily of cations and simple sugar acids. These combined properties could synergistically modify numerous controls on soil-water balances.

In a laboratory setting, we compared LB stabilized with various cations ( $K^+$ ,  $NH_4^+$ , and  $Ca^{2+}$ ) across a range of soil types to determine LB effects on soil moisture and SOC retention. All LB amendments increased soil water content relative to unamended soil across a range of soil matric potentials and raised available water content by 37%. Additionally, LB amended soils had on average 70 times more microbial biomass and decreased soil inorganic nitrogen content compared to unamended soils. We found that  $K^+$ -LB, the most effective amendment, increased soil water content by 100–600% compared to unamended soils and as much as 87% of the increased SOC following LB additions was retained after 2 months. Our results suggest that tapping into novel sources of organic inputs such as LB may be an effective approach for simultaneously enhancing soil moisture and carbon stocks while increasing the economic and energetic value of food production byproducts.

## 1. Introduction

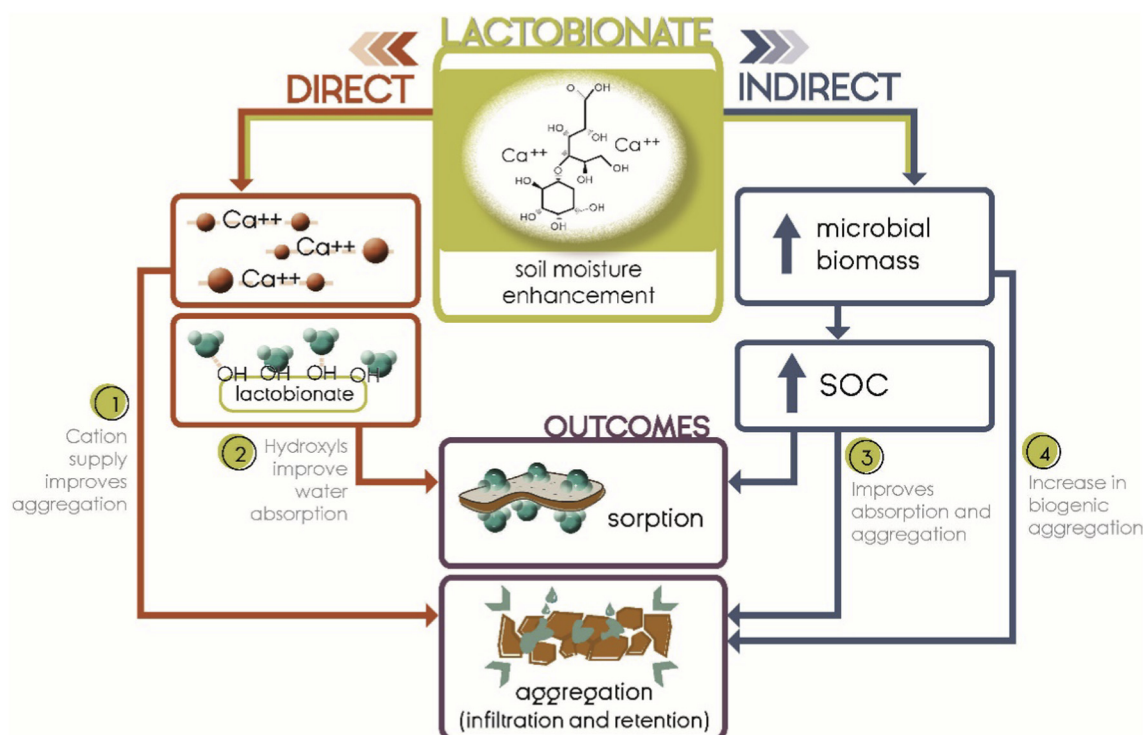
Crop water stress resulting from dry soils is common, and a key reason that crops rarely approach their genetic yield potential. At the same time, soil moisture limitations in agroecosystems will be aggravated by climate change-driven increases in drought frequency and magnitude in many regions, and irrigation aquifers are being drawn down at unsustainable rates (Ko et al., 2012; Scanlon et al., 2012). Increased climate variability will further destabilize dryland crop production and drive an overall spatial expansion of dryland agriculture (Quinn et al., 2001; IPCC, 2014; Huang et al., 2016). Water-limited agricultural systems are not only vulnerable to reduced crop yields but are often characterized by low concentrations of soil organic matter (SOM) and carbon (SOC) (O'Brien et al., 2010; Robertson et al., 2017). Given that plant inputs are an important source of SOC, depleted soil C stocks often follow soil water limitations if reductions in crop

productivity occur (Plaza-Bonilla et al., 2015). Since SOC is a proximate control on soil moisture, soil water retention may thus be further reduced, exacerbating an already water-limited system (Franzuebbers, 2002; Pimentel et al., 2005).

Soil water retention can often be enhanced through the maintenance of crop residues and the addition of amendments including manure, compost, biochar, or engineered gels (Lotter et al., 2003; Narjary et al., 2012; Omondi et al., 2016; Głab et al., 2018). However, the supply of organic amendments like manure is concentrated in animal production regions and are expensive to transport (Araji et al., 2001). Moreover, many agricultural systems (especially under dryland production) are limited by the amount of available crop residue biomass and too much manure can have unwanted environmental consequences (Singh et al., 2017; Rosenzweig et al., 2018). Other options are expensive, or have limited effects on soil moisture, making for a poor return on investment to farmers at current water prices (Minasny

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**Fig. 1.** Depiction of potential direct and indirect impacts of lactobionate on outcomes relevant for soil water content. Lactobionate may directly increase water sorption and aggregation via: 1) cation supply and 2) presence of hydroxyl groups, or indirectly via: 3) increases in soil organic C (SOC) which would support greater aggregation and water sorption, and 4) stimulated microbial activity that could result in higher SOC retention as well as biogenic aggregation through microbial polymeric exudation.

and Mcbratney, 2017). Thus, there is a clear need for new scalable technologies that can enhance soil moisture retention.

The positive effects of amendments on soil moisture are driven partly by subsequent increases in SOC, altering soil structure (e.g., promoting aggregation, modifying pore size), and because of SOC's own water adsorbing capacity (Franzuebbers, 2002; Rawls et al., 2003; Yang et al., 2014; Manns et al., 2016). While higher SOC concentrations typically correspond to improved soil structure, the relationship between SOC and soil water content is highly variable and indeed is not always positive (Rawls et al., 2003; Minasny and Mcbratney, 2017). This may be due to the numerous aspects of soil-water interactions, some of which are independent of SOC. At higher moisture levels, water movement is capillary, driven by pore size and distribution (Or and Tuller, 1999). This regime is likely where SOC will have the greatest impact on soil water (Yang et al., 2014; Karup et al., 2017). In drier soils, soil-water interactions are more a function of sorption and hydration, typically of cations and hydrophilic hydroxyl groups, directly around or within soil particles (Khorshidi et al., 2016). Both these regimes characterizing soil-water interactions can occur simultaneously at intermediate moisture levels (Lu and Likos, 2004; Frydman and Baker, 2009; Khorshidi and Lu, 2017). Novel sources of organic amendments that can capitalize on both soil-water regimes may provide opportunities to increase SOC, while simultaneously enhancing water sorption and particle hydration.

Food waste and food production byproducts are an underutilized resource for novel soil amendments. Even as concerns over agricultural water use mount, we continue to waste a large fraction of the food that is produced. Globally, one third of edible food is lost or wasted, with about 15% lost during food processing (Gunders, 2012; Rutten, 2013). For example, lactose is a major dairy industry byproduct with a global estimated 1.2M tons produced annually, much of which is wasted (Kowalczyk et al., 2007). The global surplus of lactose has led to the development of value-added lactose derivatives such as lactobionate (LB) but has not yet been evaluated for use in agricultural systems.

While diverting food processing byproducts back into agricultural fields may not directly increase calories available for human consumption, it could help close high yield gaps in particularly stressed production systems such as dryland and drought-prone cropping systems.

Here we assess the use of LB as a potential soil amendment for increasing soil moisture. Lactobionate is a unique amendment in that it may modify both capillary and adsorbed water (Fig. 1). Lactobionate is an acidic sugar (gluconic acid and galactose) extracted from whey, which is then stabilized with various cations such as calcium (Ca<sup>2+</sup>) or potassium (K<sup>+</sup>). Due to its five hydroxyl groups and one polar carboxyl, it is also a highly hydrophilic molecule that participates in hydrogen bonding, a key mechanism in aggregating soil particles and stabilizing soil C (Gutiérrez et al., 2012). Additionally, the cations associated with LB may be a key property in enhancing both capillary and adsorbed soil water regimes.

In drier soils, the adsorption of water molecules around soil particles is regulated primarily by cation abundance and their hydration (Khorshidi et al., 2016). Soil cations can also directly affect soil structure and aggregation, which are key controls on soil capillary water retention (Guber et al., 2003). The interactions between positively charged cations with negatively charged clay surfaces hold clay particles together, facilitating aggregate formation. For example, it is well known that Ca<sup>2+</sup> is effective at flocculating clays to help form soil aggregates, improving water infiltration and water movement (Kögel-Knabner et al., 2008; Rowley et al., 2018). As aggregate formation is facilitated by the presence of cations, increased SOC retention may follow. In numerous cases, Ca<sup>2+</sup> concentrations are strongly related to increases in soil C content, and often more so than clay content in dry soils (Fornara et al., 2011; O'Brien et al., 2015; Rasmussen et al., 2018). Moreover, both monovalent and polyvalent cations facilitate chemical protection of SOC through hydrogen bonding and electrostatic bridging (Rowley et al., 2018), further protecting SOM from microbial breakdown (Whittinghill and Hobbie, 2012). Thus, the hydrophilic property of LB and its associated cations may facilitate water sorption directly to

**Table 1**

Experimental treatments and soil site characteristics. Soil water holding capacity (WHC), total organic carbon (SOC), soil pH, and total nitrogen (TN) for soil sites with comparatively high soil C (HC) and low soil C (LC).

Soil site $n = 2$	San Joaquin, CA (SJ) fine sandy loam		Akron, CO (AK) silty loam	
Soil C $n = 2$	High C (HC)	Low C (LC)	High C (HC)	Low C (LC)
SOC (%)	1.02	0.29	1.78	0.84
TN (%)	0.12	0.04	0.19	0.12
pH	6.77	7.31	7.38	8.29
WHC (%)	0.204	0.22	0.32	0.26
Management	Cover crop; reduced tillage	Winter fallow; intensive tillage	Non-eroded, grassland	Mechanically eroded

soil particles as well as improve soil structure through aggregate formation and SOC retention (Fig. 1).

Indirect increases in water storage following LB amendments may also occur due to subsequently higher SOC, specifically through elevated microbial activity (Fig. 1). Unlike more common food production by-products such as chemically complex olive mill pulp and grain meals, LB is derived from simple sugar molecules. Soil microbes source much of their energy needs from plant sugars and other low-molecular weight compounds, often in limited supply, which they metabolize to build and grow their own cellular biomass (Ladd et al., 1995; Gunina and Kuzyakov, 2015). While LB may be quickly metabolized by the soil microbial community, the microbial biomass that is supported by such sugars is a primary input to SOC pools associated with clay minerals (Gleixner et al., 1999; Bradford et al., 2013; Kallenbach et al., 2015). Amending soil with LB could stimulate microbial biomass production that may then be transferred to SOC. Moreover, increases in aggregation may occur, through elevated exudation of extracellular polysaccharides with stimulated microbial growth (Deng et al., 2015; Cosentino et al., 2006). Substantial increases in microbial biomass may have subsequent impacts on nutrient cycling, especially if the microbial community becomes nitrogen (N) limited following a large pulse of sugar-derived C inputs, such as LB. While large inputs of LB stabilized with cations such as  $\text{Ca}^{2+}$  and  $\text{K}^{+}$  might immobilize N, the use of ammonium ( $\text{NH}_4^{+}$ ) as a stabilizing cation can potentially meet or exceed microbial N demands. Thus, changes in SOC and water retention with LB amendments need to be considered in conjunction with the potential impacts on soil N status.

Given the unique properties of LB as a supply of hydrophilic compounds, cations and sugar molecules, LB may directly and indirectly enhance soil water retention. Direct effects could occur from its hydrophilic and sorption properties or by increased chemical bonds that improve soil structure (Fig. 1). Indirect increases in soil moisture may also follow if biological activity is enhanced, thus increasing SOC storage and aggregate formation. In a laboratory experiment, our objectives were to: 1) evaluate formulations of LB stabilized with different cations ( $\text{Ca}^{2+}$ ,  $\text{K}^{+}$ , and  $\text{NH}_4^{+}$ -LB) over time for effects on both soil water and SOC retention; 2) assess the consequences of LB additions on soil inorganic N and microbial biomass; and 3) to identify soils differing in texture and initial SOC that have the greatest potential to respond to LB inputs. If effective, the use of LB as a soil amendment could be a promising approach for developing an alternative strategy for diverting food byproducts towards meeting soil health and crop productivity objectives.

## 2. Materials and methods

### 2.1. Field soils

In March 2016, we obtained field soils from two sites for use in our lab incubation. Soils were collected to a 20-cm depth from the USDA-ARS San Joaquin Valley Agricultural Sciences Center (Parlier, CA, USA) and from the USDA-ARS Central Great Plains Research Station Akron, CO. The San Joaquin soils (SJ) are a Hanford series fine sandy loam soil

(mixed, superactive, nonacid, thermic Typic Xerorthents) and the Akron, CO soils (AK) are a silty loam Weld series (Calderón et al., 2015) (Table 1). Mean annual precipitation in San Joaquin Valley, CA and Akron, CO is 11.5 and 16.0 in., respectively. At both locations, we collected soils from two adjacent fields that represent either a relatively low (LC) or high C (HC) soil (Table 1). The two SJ soils have both been under conventional agricultural cultivation practices for the region for > 10 yrs. The SJ-HC field (1.02% SOC) has been under reduced tillage and has a cover crop planted in the winter and terminated in the spring, while the SJ-LC field (0.29% SOC) has a winter bare fallow and receives conventional tillage operations. The AK-LC soil (0.84% SOC) was obtained from a field that had the top soil mechanically eroded down to the B horizon in 1956 and has since been cultivated and planted in winter wheat, Russian wild rye, switchgrass, and Sudan grass (Greb and Smika, 1985). The AK-HC (1.78% OC) soil was collected from a non-eroded adjacent grass field dominated by Blue grama (*Bouteloua gracilis*) and Buffalo grass (*Buchloe dactyloides*). The sites were chosen for this study for their adjacently located high and low C soils and their susceptibility to drought and low soil moisture during the summer growing season. Soils were shipped to Colorado State University at field-moisture level. Soil subsamples were oven-dried at 100 °C and analyzed for initial total organic C, N, and carbonates, and water holding capacity (WHC), or the amount of water held in the soil at field capacity (Table 1).

### 2.2. Experimental setup

Field-moist soils ( $n = 4$ ) were passed through a 2 mm-mesh sieve to homogenize samples and to remove large (> 2 mm) surface and belowground organic material. The experimental setup consisted of three lactobionate amendments with different stabilizing cations (ammonium:  $\text{NH}_4$ -LB; calcium:  $\text{Ca}$ -LB; or potassium:  $\text{K}$ -LB;) plus a control. These were combined in a full factorial with the four soils (SJ-HC, SJ-LC, AK-HC, and AK-LC) and three destructive sampling time points. All treatments were replicated three times for a total of 144 sample units.

To prepare the incubation treatments, 200 g of air-dried soil was mixed with the amendment and then brought up to 45% of WHC. Each LB amendment was added at a rate of 0.0169 g of dry LB-C g<sup>-1</sup> dry soil (0.042 g LB g<sup>-1</sup> dry soil), increasing initial soil C concentrations by 1.69%. From the 200 g of amended soil mixture, we partitioned 20 g dry weight soil into individual 50 mL polypropylene centrifuge tubes, covered with parafilm and incubated at a constant 25 °C temperature until sampling. The incubation samples were randomized within sampling time blocks. Soil moisture was checked twice weekly and readjusted to 45% WHC if necessary. Samples were destructively harvested at 2 weeks, 1 and 2 months from the start of the incubation.

### 2.3. Laboratory analyses

For each destructive sampling at 2 weeks, and 1 and 2 months, we obtained soil moisture retention curves along with soil microbial biomass C (MBC), ammonium ( $\text{NH}_4^{+}$ ) and nitrate ( $\text{NO}_3^{-}$ ) concentrations, and total soil C and N. After the incubated soils were harvested at their

prescribed times, we generated soil drying water retention curves by measuring soil matric potential ( $\psi$ ) with a WP4C dew point potentiometer (Decagon Devices; Pullman, WA), calibrated with a 0.5 M KCl solution (Scanlon et al., 2002). We began  $\psi$  measurements at in situ incubation moisture levels ( $\sim 45\%$  WHC) and collected a minimum of 6  $\psi$  measurements while the soil water was allowed to evaporate (air-drying) a (Fig. S1). Soils were immediately weighed after each  $\psi$  measurement to later determine gravimetric water content (GWC). GWC was determined for each  $\psi$  measurement based on the difference between wet and oven-dried soil weight.

To assess MBC,  $\text{NH}_4^+$  and  $\text{NO}_3^-$ , we extracted soils with 0.5 M  $\text{K}_2\text{SO}_4$  within three days of sampling. Following extraction, samples were stored at  $-20^\circ\text{C}$  until analyzed for total dissolved organic C (TOC) or inorganic N. We used the chloroform-fumigation extraction method for determining MBC, calculated as the difference between fumigated and unfumigated TOC (TOC-L CSH/CSN; Shimadzu; Kyoto, Japan) (Wu et al., 1990). Data for MBC at 2 months were not available. Soil  $\text{NH}_4^+$  and  $\text{NO}_3^-$  was determined from unfumigated  $\text{K}_2\text{SO}_4$  extracts on an Alpkem Flow Solution IV Automated Chemistry Analyzer.

On a subset of soils (SJ-HC and SJ-LC control and amended with K-LB soils), determined the amount of LB remaining in the soil at 2 weeks and 2 months was determined using capillary electrophoresis (CE) at Leprino Foods Company (Denver, CO). Samples were prepared as soil slurries of deionized water, centrifuged at 6000 RPM for 10 min, and then filtered through a  $0.22\ \mu\text{m}$  filter. Internal sucrose and LB standards were spiked into samples. Sample analysis was done on a PA800 plus CE system (System Gold software 32 KaratTM Version 9.0) (Beckman, Fullerton, CA, USA), assembled with an uncoated fused-silica capillary (SimplusTM) 70 cm in length and  $50\ \mu\text{m}$  ID (MicroSolv Technology Corporation, Leland, NC, USA). Samples were run with a maximum current of  $300\ \mu\text{A}$ , a constant voltage (25 kV) and temperature ( $20^\circ\text{C}$ ), and a data rate of 4 Hz. Peak areas of the samples were calculated against the given standard calibration curves to obtain concentration values of LB and lactose in the samples.

## 2.4. Data analyses

To statistically compare GWC values for a range of  $\psi$  values across experimental treatments, we applied non-linear model fitting using observed  $\psi$  and GWC values from the soil drying retention curves generated for each sample (Fig. S1) (Brye, 2003). This allowed us to estimate  $\psi$  as a function of GWC and to model the continuous GWC response to changes in  $\psi$ . For each sample, we entered observed  $\psi$  and GWC into the power function:  $y = ax^b$ ; where  $y$  is  $\psi$  (in MPa) and  $x$  is GWC. Model output was considered acceptable if the square residuals were  $< 3$ . A model was generated for each sample that then allowed us to derive GWC for an a priori determined range of  $\psi$  ( $-0.5$ ,  $-0.75$ ,  $-1.0$ ,  $-1.5$ ,  $-2$  and  $-3$ ). We estimated plant available water content (AWC) after curve fitting, as the difference between the GWC at  $-0.5$  and  $-1.5$  MPa. Typically, AWC is determined as the difference in GWC between field capacity (ca.  $-0.033$  MPa) and wilting point (ca.  $-1.5$  MPa), however the starting in situ moisture content in our soils was often below field capacity and thus we could not generate robust model estimates of  $\text{GWC} < -0.3$  MPa.

Modeled GWC, soil MBC, inorganic N, and total soil C and N were analyzed using a linear mixed-model three-way analysis of variance (ANOVA) where replicate was used as a random effect and site (SJ and AK), initial soil C (HC and LC), and amendment ( $\text{NH}_4\text{-LB}$ , Ca-LB, K-LB, control) were treated as fixed effects. Interactions among fixed effects were initially included in the model but if not significant ( $p > 0.05$ ) were removed and the model was reanalyzed. Pearson correlation analysis was used to evaluate relationships between soil moisture, SOC and MBC. Differences in means were determined by Tukey's HSD and considered significant if  $p < 0.05$ . All variables were analyzed within separate sampling time points.

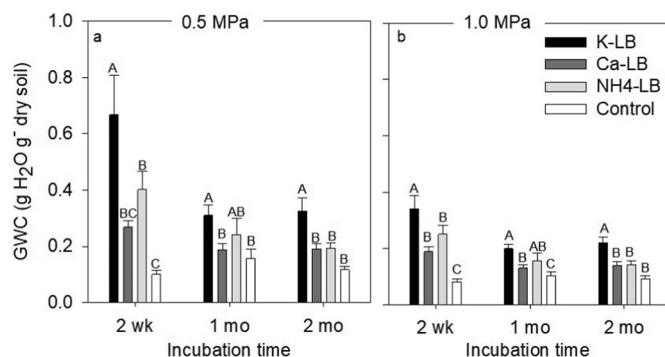


Fig. 2. Soil gravimetric water content (GWC) at (a)  $-0.5$  and (b)  $-1.0$  matric potential (MPa) at 2 weeks, 1 and 2 months following amendment additions. Differences in moisture content between amendment treatments within sampling times are indicated by different letters. Error bars are standard error;  $n = 6$ .

## 3. Results

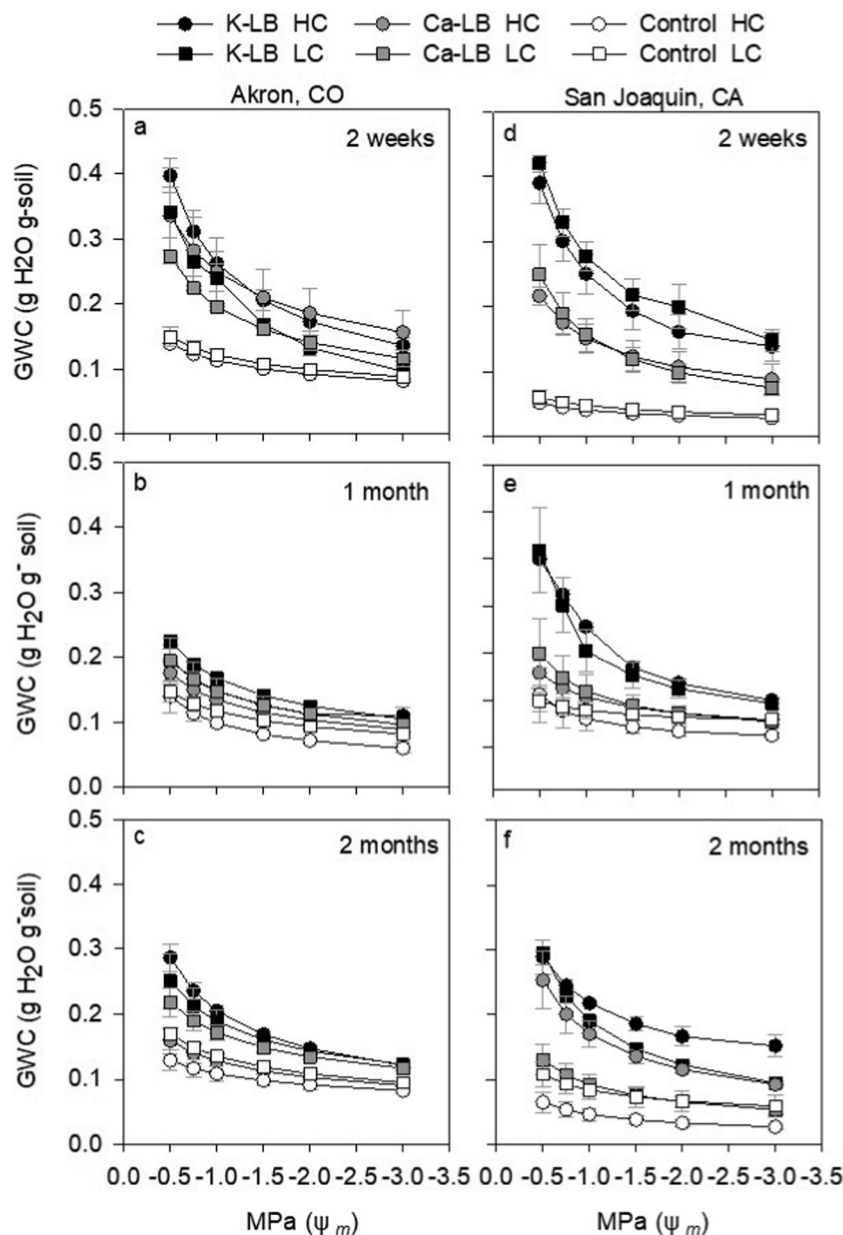
### 3.1. Soil moisture and water retention

Soils amended with LB had overall higher soil moisture across a range of  $\psi$  compared to control soils (Fig. 2; Table S1) ( $p < 0.001$ ). This effect generally persisted throughout the 2-month incubation period. Soil gravimetric water content (GWC) was highest for K-LB followed by  $\text{NH}_4\text{-LB} > \text{Ca-LB} > \text{control}$  both for wetter ( $-0.5$  MPa) and drier soils ( $-1.0$  MPa). The positive effect of LB was highest after 2 weeks and declined by 1 month (Figs. 2 and 3). However, there was no difference in the magnitude of the amendment effect on soil moisture between 1 and 2 months. Only the K-LB amended soils had consistently positive effects on soil water retention relative to the control soils throughout the incubation period and across soil  $\psi$ . At 2 weeks, when the strongest effect was observed, K-LB resulted in nearly a seven-fold increase in soil moisture (additional  $0.57\ \text{g water g}^{-1}$  soil) relative to control soils, declining to a 3-fold (additional  $0.21\ \text{g water g}^{-1}$  soil) increase after 2 months. Plant available water content (AWC) was higher in all LB treatments at 2 weeks relative to control soils, though only K-LB demonstrated relatively higher AWC throughout the incubation (Fig. 4). Relative to control soils, AWC between sites increased the most in San Joaquin soils but we observed no effect of initial SOC on AWC.

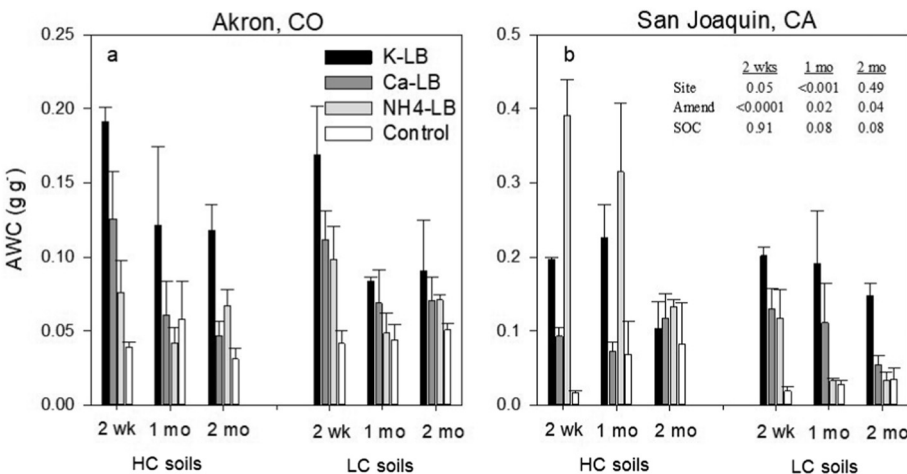
The observed increases in soil moisture due to LB additions also varied by soil  $\psi$ , where the difference between amended and control soils was greater in wetter soils and declined at lower  $\psi$  (more negative) (Figs. 2 and 3). The effect of LB on soil water retention only marginally depended on site and initial soil C concentrations (Table S1), where some interactive effects on GWC occur between site, SOC, and amendment. For example, after 2 months the soils with initially higher SOC within a site exhibited a stronger positive response to K-LB amendments compared to low SOC soils (Fig. 3). However, even though initial SOC concentrations were lower in the SJ soils, these had a stronger response to LB compared to the AK soils, with nearly double the water content at 1 and 2 months in the K-LB amended soils relative to SJ control soils (Fig. 3).

### 3.2. Soil and lactobionate carbon

We examined changes to total SOC following LB amendments over the course of the incubation. Similar to soil moisture, we observed persistent effects of amendment additions on SOC, where LB resulted in elevated SOC relative to the control soils (Fig. 5; Table S2). In general, the K- and Ca-LB treatment resulted in the most elevated SOC. The LB amendment rate increased SOC in all soils by  $16.9\ \text{mg C g}^{-1}$  soil above initial SOC concentrations, resulting in an initial 2 to 6-fold SOC

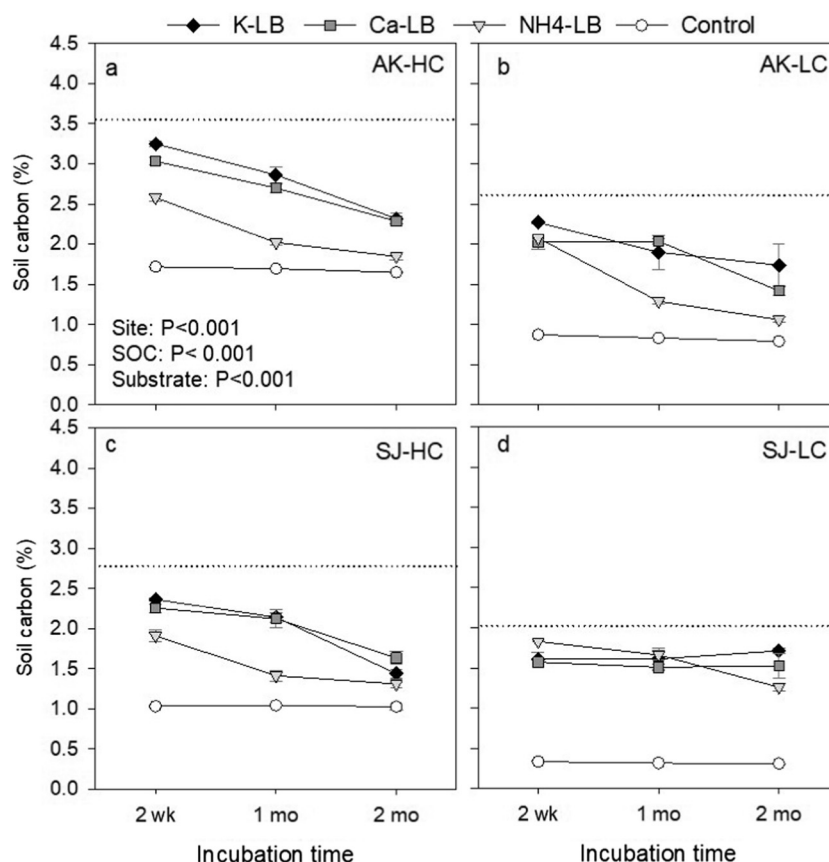


**Fig. 3.** Modeled soil gravimetric water content (GWC) across matric potentials (MPa) for (a,b,c) Akron, CO and (d,e,f) San Joaquin, CA sites for (a,d) two weeks, (b,e) one and (c,f) two months in high (HC) and low carbon (LC) soils treated with K- and Ca-lactobionate (LB) and control treatments. Error bars are standard error;  $n = 3$ .



**Fig. 4.** The plant available water content (AWC), as the water content difference between  $-0.5$  and  $-1.5$  MPa, for high (HC) and low carbon (LC) soils at 2 weeks, 1 and 2 months after amendment additions for a) Akron, CO and b) San Joaquin, CA soils. ANOVA  $p$ -values are shown for within each sampling time. Error bars are standard error;  $n = 3$ .





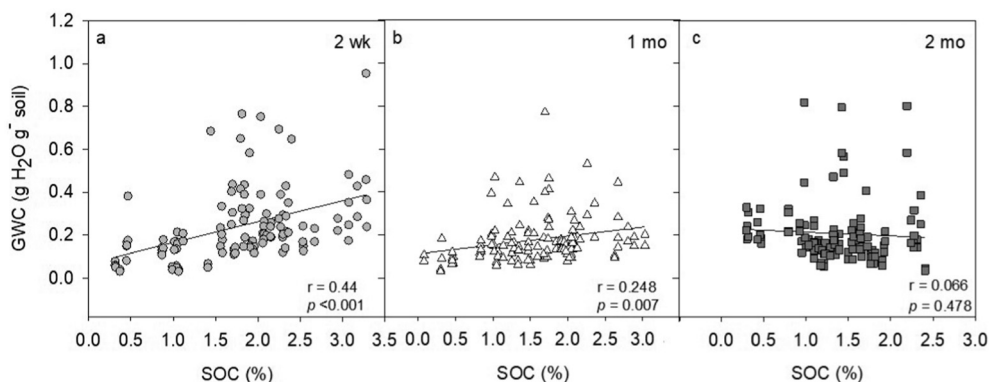
**Fig. 5.** Changes in soil organic carbon (SOC) over time for amended (K-LB, Ca-LB and  $\text{NH}_4\text{-LB}$ ) and control soils from Akron, CO (AK) high (a) and low (b) carbon soils and San Joaquin (SJ) high (c) and low (d) carbon soils. The dashed line represents %SOC immediately after amendment addition. Error bars are standard error;  $n = 3$ .

increase, depending on native SOC content. (Fig. 5).

After 2 weeks SOC concentrations were still generally 2 times higher in K-LB and Ca-LB treated soils relative to control soil and by a minimum of 1.4 times greater after 2 months. In the low C soils at 2 weeks,  $\text{NH}_4\text{-LB}$  soils showed a similar SOC response to that of the K- and Ca-LB soils. Though this did not persist and after 2 months, much more of the SOC initially gained by LB additions had been lost in the  $\text{NH}_4\text{-LB}$  soils compared to the K- and Ca-LB soils. The lower C soils for both the AK and SJ sites retained the most SOC gained following amendment additions compared to the higher C soils (Fig. 5) ( $p < 0.001$ ). This was especially pronounced in the SJ-LC soils where SOC only declined by 20% following initial K and Ca-LB additions over the 2-month period. As such, at 2 months, SJ-LC amended soils had 5

times more SOC compared to controls soils at the end of the incubation. A subset of K-LB samples were analyzed for LB remaining at 2 weeks and 2 months and we found that, despite minimal SOC declines in SJ-LC soils, no LB was detected after 2 months (Fig. S2). However, the SJ-HC soils had retained approximately 60% of the initial LB amendment.

To evaluate whether differences in SOC corresponded to the observed differences in GWC, we correlated all SOC against the estimated water content at  $-0.5$  and  $-1.0$  MPa (Fig. 6). Initially, there was a strong relationship between SOC and soil GWC, where SOC explains 44% of water content variability. However, this relationship was diminished over time such that by 2 months there is no relationship between SOC and soil moisture (Fig. 6).



**Fig. 6.** Correlations between soil water content (GWC) at  $-0.5$  and  $-1.0$  MPa and soil C (SOC) for (a) two weeks, (b) one and (c) two months.

**Table 2**

Soil microbial biomass C (MBC) by amendment, site, and initial soil organic C (SOC) of high (HC) and low (LC) soil at 2 weeks and 1 month and 3-way ANOVA *p*-values; *n* = 3.

Amendment	Site	SOC	MBC	
			(mg C kg <sup>-1</sup> soil)	
			2 weeks	1 month
K-LB	AK	HC	8349.5	7790.4
		LC	11,189.8	10,597.3
	SJ	HC	13,696.6	16,689.9
		LC	12,167.8	7277.6
Ca-LB	AK	HC	9197.3	6395.4
		LC	13,087.1	9816.2
	SJ	HC	10,362.9	11,289.9
		LC	10,133.1	7560.7
NH <sub>4</sub> -LB	AK	HC	11,037.6	869.6
		LC	9911.5	3225.4
	SJ	HC	13,627.3	18,504.8
		LC	8610.5	739.1
Control	AK	HC	193.2	156.6
		LC	188.7	150.6
	SJ	HC	233.3	138.5
		LC	17.3	20.1
ANOVA			<i>p</i> -Value	
Site			0.40	< 0.0001
SOC			0.90	0.0001
Amendment			< 0.001	< 0.0001
Amend* SOC			0.280	< 0.0001
Amend * site			0.397	0.0001
Site * SOC * amendment			0.64	< 0.0001

### 3.3. Soil microbial biomass

Regardless of LB formulation, we observed increases in soil microbial biomass C (MBC) with the addition of LB, on average 50 times greater relative to control soils (Table 2). At 1 month, MBC in LB soils had declined compared to at 2 weeks but was still 5–100 times greater relative to the unamended control soils. The exception was the SJ-HC soils, where MBC increased over time in all amended treatments. Though not consistently significant, SJ-HC soils also generally had the

highest MBC for all LB amendments and the greatest increase relative to the control soils. We did not observe any differences between NH<sub>4</sub>-, K-, and Ca-LB amendments on total MBC at 2 weeks, but at 1 month total MBC was lower in NH<sub>4</sub>-LB compared to K- and Ca-LB soils (*p* < 0.05).

### 3.4. Soil inorganic nitrogen concentrations

Lactobionate amendments only consistently influenced NH<sub>4</sub><sup>+</sup> concentrations throughout the course of the incubation under NH<sub>4</sub>-LB additions (Table 3), where NH<sub>4</sub>-LB increased NH<sub>4</sub><sup>+</sup> by 3 orders of magnitude greater than control soils. Though at 2 weeks the other LB treatments had no effect on NH<sub>4</sub><sup>+</sup> compared to control soils, NH<sub>4</sub><sup>+</sup> in both Ca- and K-LB soils increased over time to 1.5–3 ppm, to exceed control soil NH<sub>4</sub><sup>+</sup> (*p* < 0.001). Regardless of amendment, the higher C soils generally exhibited more NH<sub>4</sub><sup>+</sup> at 2 weeks and 1 month (*p* < 0.05).

Soil NO<sub>3</sub><sup>-</sup> was depleted in all LB amended soils within the first month compared to control soils (Table 3). For both K- and Ca-LB treated soils, NO<sub>3</sub><sup>-</sup> remained low (< 2.3 ppm) throughout the course of the experiment, while NH<sub>4</sub>-LB increased at 2 months to 46–200 ppm. The soil site also had a stronger influence on NO<sub>3</sub><sup>-</sup> than it did for NH<sub>4</sub><sup>+</sup>, where AK soils generally exhibited higher NO<sub>3</sub><sup>-</sup> relative to SJ soils (*p* < 0.001). This was especially pronounced in NH<sub>4</sub>-LB treated AK soils which exceeded control soil NO<sub>3</sub><sup>-</sup> by 3 times as much.

## 4. Discussion

We evaluated lactobionate (LB) as a potential soil amendment for enhancing soil water retention in drought-prone soils and show that under laboratory conditions it effectively increased soil moisture content. In conjunction with enhanced water retention, we also observed persistent elevated SOC concentrations in amended soils. While we did not evaluate specific mechanisms driving the positive moisture response to LB, we suspect that the unique traits of LB are concurrently altering SOC, soil structure, and surface sorption properties, resulting in a synergistic effect on soil water retention. Based on our results, we propose two mechanisms that explain how LB increases soil moisture. The first is related to the chemical characteristics of LB and its

**Table 3**

Soil ammonium (NH<sub>4</sub><sup>+</sup>) and nitrate (NO<sub>3</sub><sup>-</sup>) concentrations by amendment, site, and initial soil organic C (SOC) of high (HC) and low (LC) soil at 2 weeks, 1 and 2 months and 3-way ANOVA *p*-values; *n* = 3.

Amendment	Site	SOC	NH <sub>4</sub> <sup>+</sup> (μg g-soil)			NO <sub>3</sub> <sup>-</sup> (μg g-soil)		
			2 week	1 month	2 month	2 week	1 month	2 month
K-LB	AK	HC	0.58	0.17	0.86	0.17	0.13	0.21
		LC	0.41	0.11	1.29	0.17	0.22	0.09
	SJ	HC	1.74	1.07	1.39	0.04	0.24	0.09
		LC	1.31	0.19	1.28	0.19	0.23	0.08
Ca-LB	AK	HC	1.84	1.03	1.63	0.22	0.12	0.15
		LC	0.53	0.16	2.23	0.30	0.05	2.24
	SJ	HC	1.87	0.86	2.13	0.29	0.05	0.75
		LC	1.76	0.82	3.10	0.17	0.02	0.74
NH <sub>4</sub> -LB	AK	HC	159.28	219.90	135.97	0.91	0.96	199.58
		LC	159.96	226.65	117.68	1.27	0.24	154.19
	SJ	HC	188.38	282.84	123.64	0.10	2.09	46.87
		LC	144.91	125.24	142.80	0.50	0.02	88.69
Control	AK	HC	1.25	0.19	0.58	57.00	70.39	55.24
		LC	1.11	0.21	0.83	40.88	52.14	70.66
	SJ	HC	1.41	0.22	0.81	38.97	62.42	57.66
		LC	1.08	0.17	0.88	11.13	11.88	58.95
ANOVA			<i>p</i> -value					
Site			0.379	0.002	0.860	< 0.0001	< 0.0001	0.061
SOC			0.030	< 0.0001	0.9685	0.204	< 0.0001	0.560
Amendment			< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
Amend* SOC			0.002	< 0.0001	1.000	0.173	< 0.0001	0.830
Amend * site			0.734	< 0.0001	1.000	< 0.0001	< 0.0001	0.025
Site * SOC * amendment			0.004	< 0.0001	0.9385	< 0.0001	< 0.0001	0.998

associated cations and hydroxyl groups that would facilitate both aggregation formation and water sorption (Khorshidi et al., 2016; Rasmussen et al., 2018), irrespective of change in SOC. The second mechanism is driven by stimulated microbial activity, resulting in retention of the added C within microbial biomass, as well as enhanced biogenic aggregate formation (Fig. 1).

#### 4.1. Lactobionate effects on soil moisture

All of the LB amendments increased soil water content relative to unamended control soil across the range of water potentials we tested. Thus, LB appears to have the potential to increase soil water storage by reducing water loss. Specifically, we characterized the impact of LB on the relationship between soil water content and  $\psi$ . This relationship typically varies across different soils and is strongly influenced by soil structure and texture (Tarawally et al., 2004). Water potential characterizes the energy required for water to move through the soil profile or be taken up by crop roots. Relative to coarse textured soils, greater surface area and tighter pore space of fine textured clay requires more energy to move water through the profile. Consequently, we could expect greater water storage with less water lost to evaporation or drained away from the root zone in finer textured soils. However, altering soil texture is not a feasible management strategy for increasing water storage at the field-level. Due to the properties of LB which may improve soil structure and water sorption, amending soil with LB might mimic the benefits associated with finer textured soils.

The K-LB treatment was consistently the most effective at increasing water retention throughout the 2-month incubation period across the soil types and range of  $\psi$  values we tested (Figs. 2 and 3). Since  $\text{Ca}^{2+}$  is a divalent cation that binds clay particles more strongly than the monovalent  $\text{K}^+$ , we expected the Ca-LB amendment to have the strongest and most consistent influence on soil moisture. Rather, it was relatively less effective than K-LB in increasing water retention, though similar amounts of SOC were retained between K- and Ca-LB. As such, there are likely other dynamics beyond differences in chemical bonding mechanisms that resulted in the stronger soil moisture effect we observed with K-LB. In alkaline soils, such as the ones in our study, high Ca concentrations can be one of the primary pathways of phosphorus fixation, causing microbial phosphorus limitations and thus reductions in microbial activity with Ca-LB applications (Bertrand et al., 2003; McLaughlin et al., 2011). However, we did not see evidence for this, as MBC differences between K- and Ca-LB were not significant and nitrate levels (indicative of nitrification activity in this incubation) were higher in Ca-LB soils. Alternatively, the fixation of both  $\text{NH}_4^+$  and  $\text{K}^+$  within clay interlayers may be affecting how LB influences soil water dynamics in ways distinct from Ca-LB additions (Martin and Sparks, 1985; Nieder et al., 2011).

We observed the strongest effects of LB in wetter soils, especially at  $-0.5$  MPa, compared to lower  $\psi$  values, which explains why we detected overall net increases in available water (Fig. 4). If increases in soil moisture in response to LB had been similar across matric potentials, AWC would have been less affected by LB amendments (Hudson, 1994). The forces that regulate soil water retention differ as  $\psi$  declines. At or near field capacity, water retention is strongly affected by soil structure, but at lower  $\psi$  ( $< -1.5$  MPa) sorption, soil texture and surface area become more important drivers (Tarawally et al., 2004). In several cases, stronger effects of SOC on soil water retention have been observed in relatively wetter soils (Rawls et al., 2003; Yang et al., 2014), where pore size and distribution are key regulators of water movement. As such, the often-observed relationship between SOC and moisture may primarily be a function of organic matter changing soil structure, with weaker effects associated with the sorptive and surface area properties of SOC. Accordingly, the stronger positive effects we observed from LB amendments under wetter soils may have been driven more by alterations in soil structure and aggregation than by changes in the sorptive properties of the soil matrix (Frydman and Baker, 2009;

Karup et al., 2017).

The San Joaquin soils, with a relatively coarser texture and lower initial SOC, were also more responsive to LB amendments compared to Akron soils. Similar to observations by Rawls et al. (2003), the sensitivity of water retention to organic inputs declined with increasing SOC content. Many inconsistencies in SOC-water relationships have been attributed to soil texture variability, where the relationship is often stronger in coarser textured soil, also corresponding with lower SOC (Khaleel et al., 1981; Minasny and Mcbratney, 2017). Given that Akron control soils had slightly higher moisture retention, it is possible that this higher water retention ‘baseline’ constrained room for improvement with LB additions. These observed patterns, described by Minasny and Mcbratney (2017) as a diminishing returns effect, may be a result of redundancy if many of the water retention mechanisms supported by organic inputs are already activated by clay content or native SOM.

#### 4.2. Lactobionate effects on soil carbon

Lactobionate amendments increased SOC concentrations relative to control soil throughout the 2-month incubation. A substantial amount of LB-C (1.67% above baseline) was added and so it was expected that we would observe an initial increase in SOC. However, LB, a disaccharide, is theoretically an ideal energy source for many soil microbes and thus likely to be readily consumed. Nonetheless, after 2 months the initial increases in SOC from LB additions had persisted. Amended soils were on average at 67%, and as high as 87%, of the SOC obtained immediately following LB additions. As such, LB additions resulted in a maintenance of 1.5–5.5 times greater SOC compared to native SOC concentrations at 2 months. While LB may quickly be utilized by soil microbes, microbial biomass is an important C source for building persistent SOC (Kallenbach et al., 2016). Hence, a portion of the elevated SOC we observed in the LB-amended treatments likely rather represents LB conversion into microbial materials which can thus delay or potentially even halt its loss out of the system (Liang et al., 2017). Indeed, we did observe microbial biomass carbon concentrations that were 50–100 times higher in LB amended soils compared to control soils. Moreover, LB was undetectable in the low carbon SJ soils which exhibited the greatest persistent increase in SOC, relative to control soils (5-fold increase). However, the high carbon-SJ soil had 60% of the added LB at 2 months, with only a 1.5-fold increase in SOC compared to control soils. Given its high energy concentration, LB may be priming the soil community to breakdown native SOM in higher C soils. Soils with relatively more decomposable C are more likely to exhibit positive priming effects (Kuzyakov, 2002), potentially explaining why we find LB remaining in SJ-HC soils but not SJ-LC soils. In the SJ-LC, the low 0.3% initial SOC may rather be facilitating substrate switching from SOM to LB, inducing a negative instead of a positive priming effect.

The overall persistence of the SOC in the LB soils may also be a consequence of changes to soil structure that could arise from chemical properties of LB, as well as biogenic aggregation from stimulated extracellular microbial polysaccharide production (Deng et al., 2015; Blankinship et al., 2016). For example, Lehrs et al. (2008) observed a 25% increase in aggregate stability following whey applications to soil. Enhancing soil structure not only improves soil water retention but also helps to protect SOC from decomposition, with potential beneficial feedbacks on water retention and sorption. Thus, there is likely an interaction between soil moisture, structure and SOC, where the magnitude of LB effects on soil moisture may be a partial consequence of increased SOC (Fig. 1).

The soil particulate organic matter pool is considered an important SOC fraction in controlling soil aggregation and moisture retention (Christensen, 2001). However, LB would not likely contribute substantially to the particulate SOM fraction as much as plant-and manure-based amendments. In our study, there was a significant influence of SOC on water content variability after 2 weeks and 1 month of incubation (Fig. 6). Thus, other processes contributing to aggregate



formation or overlooked SOM fractions may also be affecting soil water dynamics. After 2 months, the relationship between SOC and water content disappears but we continue to observe impacts of LB on soil moisture, suggesting that the mechanisms supporting enhanced soil moisture are shifting over time towards ones that are independent of SOC.

#### 4.3. Lactobionate effects on soil inorganic N

Given the coupling of soil C and N cycling and the high rates of C inputs we added, we wanted to evaluate the effects of LB on crop-available N. If soil C concentrations relative to N are too high, microbes will temporarily immobilize N in their biomass, reducing how much is transformed into plant available  $\text{NO}_3^-$  (Cheng et al., 2017). As expected, the use of  $\text{NH}_4\text{-LB}$  resulted in  $\text{NH}_4^+$  concentrations well above the typical range for most agricultural soils (2–15 ppm). However, neither the Ca- nor the K-LB amendment influenced soil  $\text{NH}_4^+$  concentrations relative to control soils. Soil  $\text{NO}_3^-$  was relatively depleted throughout the incubation in the Ca- and K-LB soils. Given the high microbial biomass in the LB amended soils and high microbial N demand during growth, this depletion is likely because microbial biomass N immobilization is being favored over nitrification. Over time, as microbial biomass turns over, much of that N would be released into the soil and available for plant uptake. However, after 2 months we had not yet seen any change in  $\text{NO}_3^-$ , except in the  $\text{NH}_4\text{-LB}$  treated soils. The depletion of soil  $\text{NO}_3^-$  can either be a benefit or disadvantage depending on when and how long it occurs. During non-crop growing periods, reducing soil  $\text{NO}_3^-$  can be both an economic and environmental benefit by reducing off-farm inorganic losses and ground water pollution (Basso et al., 2016). If, however,  $\text{NO}_3^-$  is depleted during the peak growing season when crop N demand is high, yields can suffer.

#### 5. Implications and conclusions

The potential for LB to synergistically support multiple pathways of water retention, may explain why the soil moisture improvements we observed with LB are higher than previously reported for other forms of organic amendments (Minasny and Mcbratney, 2017). In their synthesis, Minasny and Mcbratney (2017) estimated a mean 2.1 increase in plant available water following a 1% increase in SOC from mulches, manure, and compost additions. Our LB treatments only had a slightly higher C input rate (1.67%), but showed a 6 times higher mean AWC with LB. Using a soil bulk density of 1.1, our mean 12.96 ( $\text{g H}_2\text{O } 100 \text{ g}^{-1} \text{ soil}$ ) AWC with LB would translate to an estimated increase in soil water of 14.26  $\text{mm H}_2\text{O } 100 \text{ mm}^{-1} \text{ soil}$ . Under some climate change simulation scenarios, this increase in AWC has the potential to mitigate climate-change related increases in irrigation water demands (50  $\text{mm yr}^{-1}$ ) by 25% (Fischer et al., 2007).

Of the three LB amendments we evaluated, we found that the K-LB was the most effective and most consistent in its influence on both soil water retention and SOC. While we cannot extrapolate our findings beyond the experimental period, the persistent 2-month effect of K-LB encompasses the duration for most critical crop development stages. These results provide a proof-of-concept regarding the effectiveness of LB and potentially other similar food production byproducts such as whey or molasses on enhancing moisture retention in drought-prone soils. The sustained elevated increases in SOC we observed may not only benefit soil moisture retention but are also critical to regenerating soil health. Governmental agencies worldwide are instituting initiatives which encourage land managers to emphasize practices that build SOC (Bünemann et al., 2018). For example, California launched the Healthy Soils Initiative and Action Plan in 2016, aimed at providing financial incentives to growers that implement soil management practices that reduce drought risk and increase soil C sequestration (CDFA, 2017). Our results suggest that tapping into novel sources of organic inputs such as lactobionate may be an effective approach for simultaneously

ameliorating drought affected soils and increasing soil C stocks while increasing the economic and energetic value of food production by-products.

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#### Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.geoderma.2018.09.027>.

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